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GROWTH OF ANIMAL CELLS SUSPENDED IN SERUM-FREE MEDIA: II. FERMENTOR CULTURES

Stanley C. Nagle, Jr.

Bruce L. Brown

Gordon Taylor

Medical Bacteriology Division
DIRECTOR OF BIOLOGICAL RESEARCH

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ABSTRACT

Growth of cat kidney, HeLa, and L cells was obtained in 4-liter volumes of a chemically defined medium in 7.5-liter fermentors. Viable cell counts of 23×10^8 to 33×10^8 per culture were recorded after 11 to 13 days of incubation from inocula of 1×10^8 to 4×10^8 cells. Under similar conditions, cultures of L cells in serum-free lactalbumin hydrolyzate medium yielded 34×10^8 viable cells in 7 days. L cells grown in 14-liter fermentors containing 10 liters of serum-free lactalbumin hydrolyzate medium yielded a total viable count of 14×10^9 cells (2 tanks) from an inoculum of 56×10^7 cells after 16 days of incubation.

I. INTRODUCTION

Growth of established animal cell lines in large-scale suspension cultures was reported by McLimans et al.[1] using a 5-liter fermentor, by Ziegler et al.[2] in a 20-liter fermentor, and by Rightsel et al.[3] in a 30-liter fermentor. Media employed by these workers in fermentor assemblies contained serum. In a recent report, Merchant et al.[4] described the growth of L-M cells in 40 liters of serum-free Medium 199 supplemented with peptone in a 100-liter fermentor. The present report describes the growth of cat kidney, HeLa, and L cells in a chemically defined medium in a 7-liter fermentor, and growth of L cells in a serum-free lactalbumin hydrolyzate medium in 7- and 14-liter fermentors.

II. MATERIALS AND METHODS

A. MEDIA AND CELLS

The composition and preparation of the chemically defined and lactalbumin hydrolyzate media, and the characterization of cell lines and counting procedures, were previously described by Nagle et al.[5] and Nagle.* By our tests all cells were judged free of pleuro-pneumonia-like organisms (PPLO).

B. SEVEN-LITER FERMENTOR SYSTEM

The New Brunswick fermentor stand variable-drive assembly** was used for these studies. The incubation temperature was 35 C. Cells were kept in suspension during growth with dual impellers placed at 0 and 12 cm from the bottom of the agitator shaft and operated at 135 to 150 rpm. The upper impeller made contact with the culture after the medium volume was adjusted to 3 liters. Cultures were not aerated. Growth was initiated in 1 liter of medium in 7.5-liter glass fermentor tanks. The inoculum prepared from rotary-shaken cultures yielded an initial viable cell count of 1×10^5 to 4×10^5 cells per ml. When the cell population reached 6×10^5 to 8×10^5 cells per ml, the culture was centrifuged, resuspended in 2 liters of fresh medium, and reincubated. This process of removing 1 liter of spent medium and replacing with 2 liters of fresh medium was repeated until a total volume of 4 liters was reached.

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* Nagle, S.C., Jr. 1964. Growth of animal cells in suspension in serum-free media: I. Studies with insulin in defined medium. To be published.

** Model FS 307, New Brunswick Scientific Co., New Brunswick, N.J.

C. FOURTEEN-LITER FERMENTOR SYSTEM

A New Brunswick fermentor assembly with 14-liter glass tanks was employed in these studies. Incubation temperature and location of the dual impellers were the same as previously described. The agitator shaft was operated at 200 rpm. Growth was initiated in 4 liters of medium inoculated from rotary-shaken cultures. The initial count was 1.4×10^5 viable cells per ml. When the viable count approached 6×10^5 to 8×10^5 cells per ml, 2 liters of fresh medium were added. The culture volume was increased in this manner until a final volume of 10 liters was obtained.

III. RESULTS

A. GROWTH OF CAT KIDNEY, HELA, AND L CELLS IN THE 7.5-LITER FERMENTOR

Growth curves of cat kidney, HeLa, and L cells were determined. Average results of replicate cultures of each cell line are shown in Figure 1. The highest total viable cell yields (approximately 33×10^8) were obtained with L cells at 7 and 11 days in lactalbumin hydrolyzate and chemically defined medium respectively. Maximal yields of cat kidney and HeLa cells were obtained in chemically defined medium at 12 and 13 days respectively. Several attempts to obtain satisfactory yields of cat kidney or HeLa cells in the lactalbumin hydrolyzate medium in the fermentor have failed.

B. GROWTH OF L CELLS IN THE 14-LITER FERMENTOR

Results of this experiment are shown in Figure 2. Note that the original 4-liter volume of lactalbumin hydrolyzate medium was divided at 5 days to give duplicate tanks. Each tank was then increased in volume by 2-liter increments as the count approached 7×10^5 to 8×10^5 cells per ml. The data plotted in Figure 2 represent the average viable count per ml for the 2 tanks and the total viable count produced in the 2 fermentor tanks during the 5- to 16-day period of incubation. The data for the first 5 days represent growth in the original tank before the culture was divided.

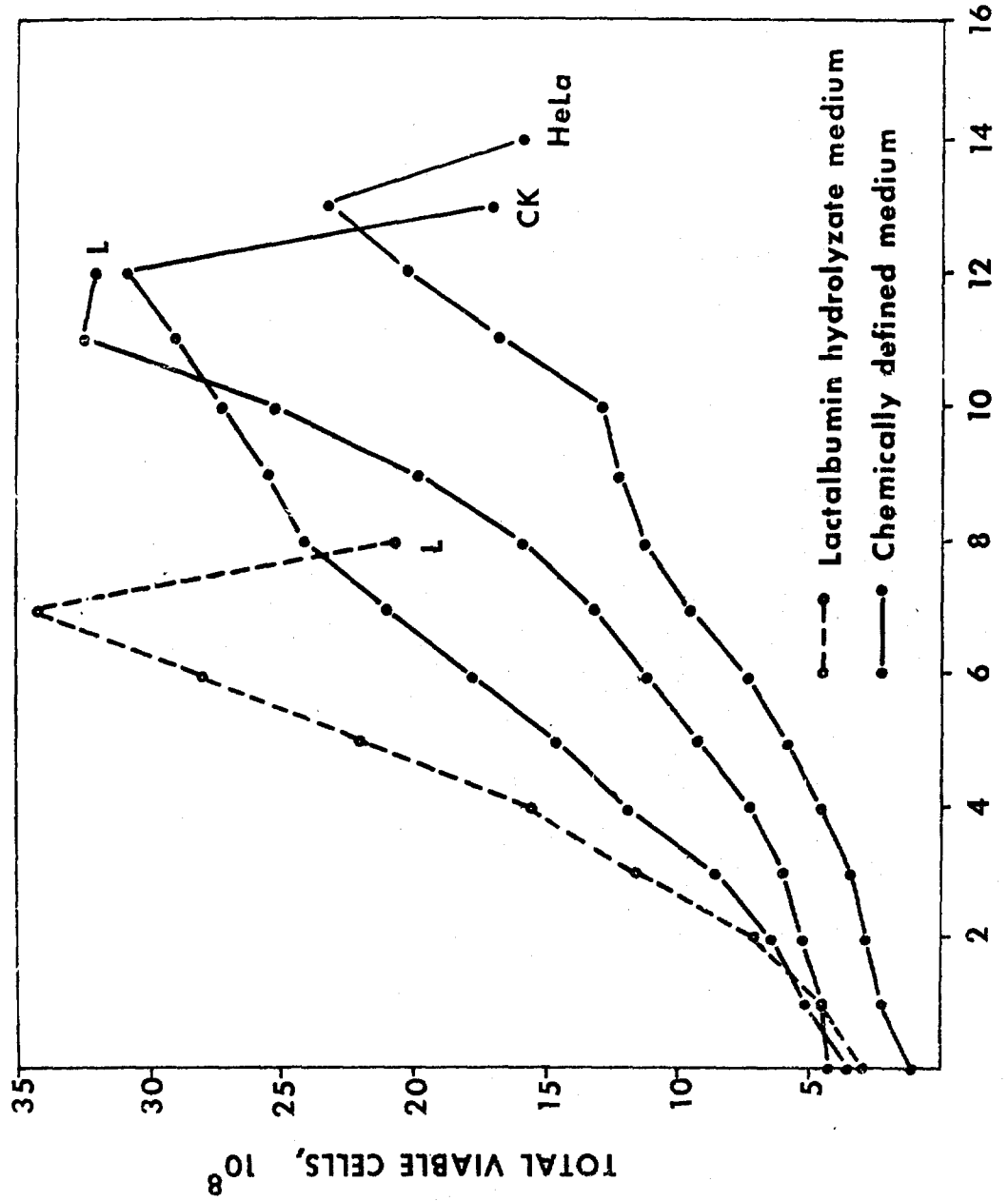

Figure 1. Growth of cat kidney (CK), HeLa, and L cells in the 7.5-liter fermentor.

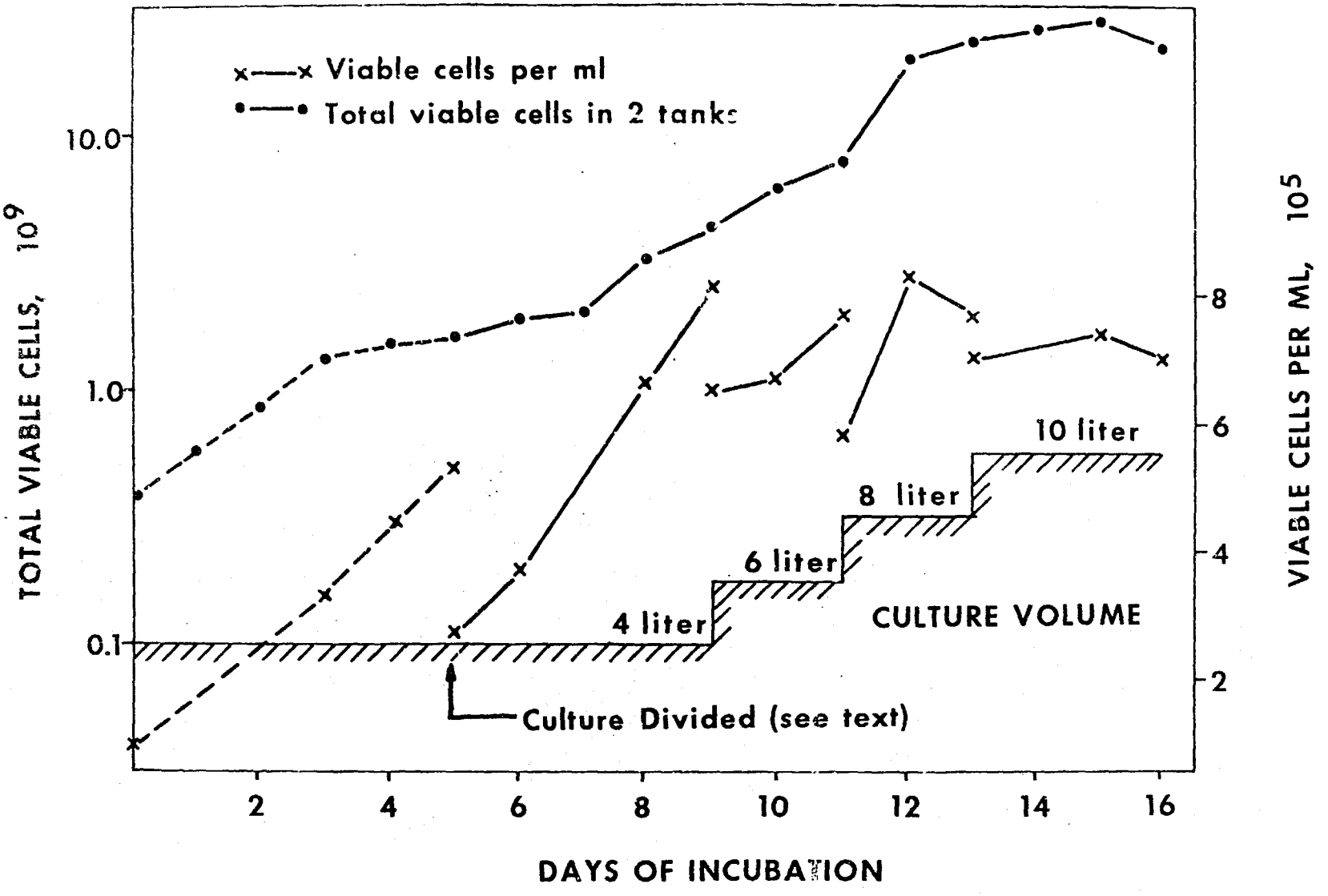

Figure 2. Growth of L cells in lactalbumin hydrolyzate medium in the fermentor.

IV. DISCUSSION

The results show that standard New Brunswick fermentor assemblies may be employed for the large-scale growth of animal cells in serum-free media. Although cell populations in the fermentors rarely exceeded 9×10^5 per ml, cell populations in identical media in serum bottles, incubated on a rotary shaker, routinely reached 3×10^6 to 5×10^6 per ml. The reduced growth in fermentors may be explained in part by the fact that cells in the fermentor did not receive the frequent complete medium changes that were routine in shaker cultures. The inhibitory effect of the accumulation of spent medium was apparent in the 14-liter fermentor. As the volume was increased, reduced growth (cells per ml) was noted after 9 days of incubation. Another factor to be considered is that microscopic examination frequently revealed cellular damage, probably caused by the shearing action of the fermentor impellers or baffle plates. This was particularly apparent in the 14-liter fermentor at 200 rpm.

The inability of HeLa and cat kidney cells to grow in fermentors in lactalbumin medium cannot be satisfactorily explained. Since these cells do grow in fermentors in chemically defined medium, differences in medium may be considered. In this regard it is possible, but not proved experimentally, that some inhibitory substance may be present in lactalbumin hydrolyzate. Failure of growth of HeLa and cat kidney cells in lactalbumin medium in the 7-liter fermentor precluded any attempt to grow these cells in the 14-liter fermentor.

Sparging or unusual buffering systems were not employed. Toward the end of the growth curves the pH tended to decrease, and perhaps this contributed also to cessation of growth. This problem was recognized by McLimans et al.[1] Their serum medium, however, received some buffering from serum components and could also be manipulated by varying the concentration of the phosphate buffer. Increasing quantities of phosphate rapidly become toxic in the serum-free media, but other buffering systems may prove effective.

The possibility exists that large-scale suspension cultures may be continuously fed and toxic metabolites reduced by utilizing a medium reservoir circulated through a dialysis feeding system. Such a system may substitute for the centrifugation and medium-replacement procedures that are quite satisfactory with smaller volumes of culture. A preliminary investigation of this possibility has yielded encouraging results.

Merchant and Eidam[6] have discussed the use of animal cells in large-scale culture and have pointed out some of the advantages of employing serum-free media. We have shown that a chemically defined medium, previously reported by Nagle, is capable of supporting the growth of several established cell lines (cat kidney, HeLa, L) in fermentor cultures. L cells, used by most investigators for large-scale growth studies, also grow in a serum-free lactalbumin hydrolyzate medium.

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